Remarks

Based on the currently pending claims and the following remarks, Applicants respectfully request that the Examiner reconsider and withdraw all outstanding rejections.

Claim Status

Claims 11-12, 14-15, 17-19, 59, 63-67, and 78-79 are pending in the application, with claims 11, 12, and 18 being the independent claims. Claims 1-10, 13, 16, 20-58, 60-62, 68-77 and 80-85 have been cancelled.

Rejection Under 35 U.S.C. § 102(e)

Claim 20 is rejected under 35 U.S.C. § 102(e) as being anticipated by Horn *et al.* (U.S. Patent No. 6,465,175). By way of the amendment filed September 5, 2006, under 37 CFR § 41.33(b), claim 20 has been cancelled. Thus, the rejection of this claim is rendered moot.

Rejection Under 35 U.S.C. § 103(a)

Claims 11-12, 14-15, 17-20, 59 and 63-67 are rejected under 35 U.S.C. § 103(a), as being unpatentable over Horn *et al.* (US Patent No. 6, 465,175) in view of Tyagi *et al.* (US Patent No. 6,037,130). By way of the amendment filed September 5, 2006, under 37 CFR § 41.33(b), claim 20 has been cancelled and rejection of this claim is rendered moot. With regard to remaining claims 11-12, 14-15, 17-19, 59 and 63-67, Applicants respectfully traverse the rejection for the reasons already of record as well as for the reasons set forth below.

Establishing *prima facie* obviousness requires a showing that some combination of objective teachings in the prior art and/or knowledge available to one of skill in the art would have lead that individual to arrive at the claimed invention. *See* In re Fine, 837 F.2d 1071, 1074 (Fed. Cir. 1988). The mere fact that references *can* be combined or modified does not necessarily render the resultant combination obvious. See MPEP 2143.01. For example, if the proposed combination of cited references changes the principle of operation of the cited art, then the teachings of the combined references cannot be used to render the claimed invention *prima facie* obvious. *Id* citing In re Ratti, 270 F.2d 810, 123 USPQ 349 (CCPA 1959). The Federal Court holds that a determination of *prima facie* obviousness is not supported in cases where the "combination of references would require a substantial reconstruction and redesign of the elements shown in [a cited reference] as well as a change in the basic principle under which the [cited reference] construction was designed to operate." See In re Ratti, 270 F.2d at 813, 123 USPQ at 352.

The currently pending claims are drawn to methods involving the detection of single-labeled oligonucleotides that are incorporated into synthesized nucleic acids. In particular, the invention defined by independent claim 11 relates to a method for quantifying or detecting nucleic acid molecules during nucleic acid synthesis. The method of claim 11 involves mixing a target nucleic acid with a fluorescently-labeled oligonucleotide. The oligonucleotide has a single type of fluorescent label with the same chemical structure, and undergoes a detectable change in fluorescence upon hybridization to the target nucleic acid. The mixture containing the target nucleic acid and the fluorescently-labeled oligonucleotide is incubated under conditions sufficient to synthesize a nucleic acid molecule complementary to all or a portion of the target nucleic acid whereby the labeled oligonucleotide is incorporated into the synthesized nucleic acid.

Independent claim 12 relates to a method for quantitation or detection of nucleic acid molecules during nucleic acid amplification. The method of claim 12 involves mixing a target nucleic acid with a fluorescently-labeled oligonucleotide. The oligonucleotide has a single type of fluorescent label with the same chemical structure, and undergoes a detectable change in fluorescence upon hybridization to the target nucleic acid. The mixture containing the target nucleic acid and the fluorescently-labeled oligonucleotide is incubated under conditions sufficient to amplify a nucleic acid molecule complementary to all or a portion of the target nucleic acid whereby the labeled oligonucleotide is incorporated into the amplified nucleic acid.

Independent claim 18 relates to a method for amplifying a double stranded nucleic acid. The method of claim 18 involves the use of primers that have a single type of fluorescent label with the same chemical structure and undergo a detectable change in fluorescence upon hybridization to a complementary nucleic acid. The primers are hybridized to opposite strands of the double stranded target nucleic acid in the presence of a polymerase under conditions sufficient to extend and incorporate the primers into a newly synthesized complementary nucleic acid. Amplification of the double stranded target nucleic acid occurs with repeated rounds of denaturation, hybridization and primer extension.

The Horn reference discloses a probe-based method for detecting target nucleic acids. Horn's method involves mixing target nucleic acid molecules with an oligonucleotide probe having a single fluorescent label (*i.e.,* a single-labeled probe). As the Examiner acknowledges (see Office Action dated January 4, 2006, page 6), Horn fails to teach incorporation of a single-labeled oligonucleotide into a synthesized nucleic acid product and relies on the Tyagi reference to cure this deficiency. The Tyagi reference discloses a triple-labeled molecular beacon-based method for

detecting target nucleic acids. More specifically, Tyagi teaches a pair of fluorophores on the primer or probe that are allowed to interact by fluorescence resonance energy transfer (FRET) when separated from a quencher label located on the opposite end of the primer/probe upon hybridization to a target nucleic acid. *See* Tyagi, column 8, lines 13-60.

The Office Action states that "it would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to utilize the single-label method of Horn with the hairpin primers of Tyagi." See Action on page 6. The Action goes on to state that "an ordinary practitioner is motivated to substitute the single label quenching beacons of Horn in the method of Tyagi so that a separate quenching dye is not necessary. *Id* at page 7. However, the proposed modifications to Tyagi's primers based on the single-label method of Horn's probes changes the basic principle under which Tyagi was designed to operate and therefore the combination of Horn and Tyagi is insufficient to render the claimed methods *prima facie* obvious.

If Tyagi's molecular beacon primers were modified as the Office Action suggests, (*i.e.,* "so that a separate quenching dye is not necessary"), the modification would require a substantial reconstruction and redesign of Tyagi's disclosed invention. This is because Tyagi's invention is based on the principle of wavelength shifting or fluorescence resonance energy transfer (FRET) between different fluorophores. Tyagi's invention includes primers and probes comprising: (a) a fluorescent emitter moeity; (b) a fluorescent harvester moiety; and (c) a quencher moiety. Tyagi states that the structure and operation of their modified molecular beacon probes and hairpin primers are designed such that "***two*** fluorophores [emitter and harvester] ***must*** be spaced relative to one another such that they can undergo fluorescence resonance energy transfer (FRET)." See Tyagi at column 8, lines 57-59. Tyagi goes on to state that:

> The transfer of energy from the harvester fluorophore to the emitter fluorophore is governed by the rules of fluorescence resonance energy transfer [Stryer (1979) Ann. Rev. Biochem. 47:819], which we use to aid in the design of probes and primers according to this invention. See Tyagi at column 8, lines 49-53.

Thus, when Tyagi's multi-labeled molecular beacon primers are in a hairpin conformation (i.e., "closed conformation"), fluorescence is suppressed but when the molecular beacon primers are hybridized to a target nucleic acid (i.e., "open conformation"), they undergo FRET and fluorescence becomes detectable. See Tyagi at column 2, lines 29-34.

In contrast, the general premise behind Horn's invention is that ***single-labeled*** probes "undergo a spontaneous fluorogenic conformational change when they hybridize to their target" ***without*** the use of a separate quenching dye. See Horn at column 17, Example 6. Thus, even if a skilled artisan were able to make Tyagi's molecular beacon primers single-labeled (as suggested by the Office Action), the resultant modified primer would not operate as the Tyagi invention was intended (i.e., utilizing FRET-based principles). This is because Tyagi's probes/primers have two other moieties (emitter and harvester) in addition to the quencher dye which allow Tyagi's methods to function by wave-length shifting or energy transfer methods. If Tyagi's primers were in fact made to be single-labeled, FRET-based fluorescence would not be employed and the basic principle of operation behind Tyagi's invention would be altered completely.

In conclusion, to "substitute the single label quenching beacons of Horn in the method of Tyagi so that a separate quenching dye is not necessary" as the Office Action suggests, would require changes to the basic principle of operation used in Tyagi's methods. For at least this reason (as well as those already made of record), the combination of the cited references are insufficient

and cannot be relied upon to show *prima facie* obviousness of the claimed methods. Applicants therefore request that the rejection of claims 11-12, 14-15, 17-19, 59 and 63-67 be withdrawn.

Conclusion

All of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore request that the Examiner reconsider and withdraw all presently outstanding rejections. Applicants believe that a full and complete reply has been made to the outstanding Final Office Action dated August 29, 2007 and, as such, the present application is in condition for allowance.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

Date: February 29, 2008

/Bernadette M. Perfect/

Bernadette M. Perfect
Agent for Applicants
Reg. No. 53,267

Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008
(760) 476-7120